

Exploring ECD on a benchtop Q Exactive Mass Spectrometer

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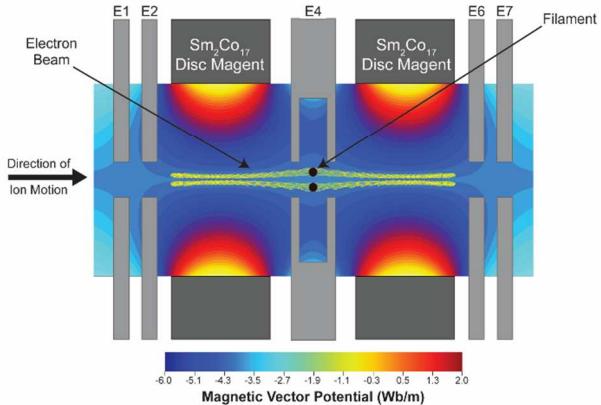
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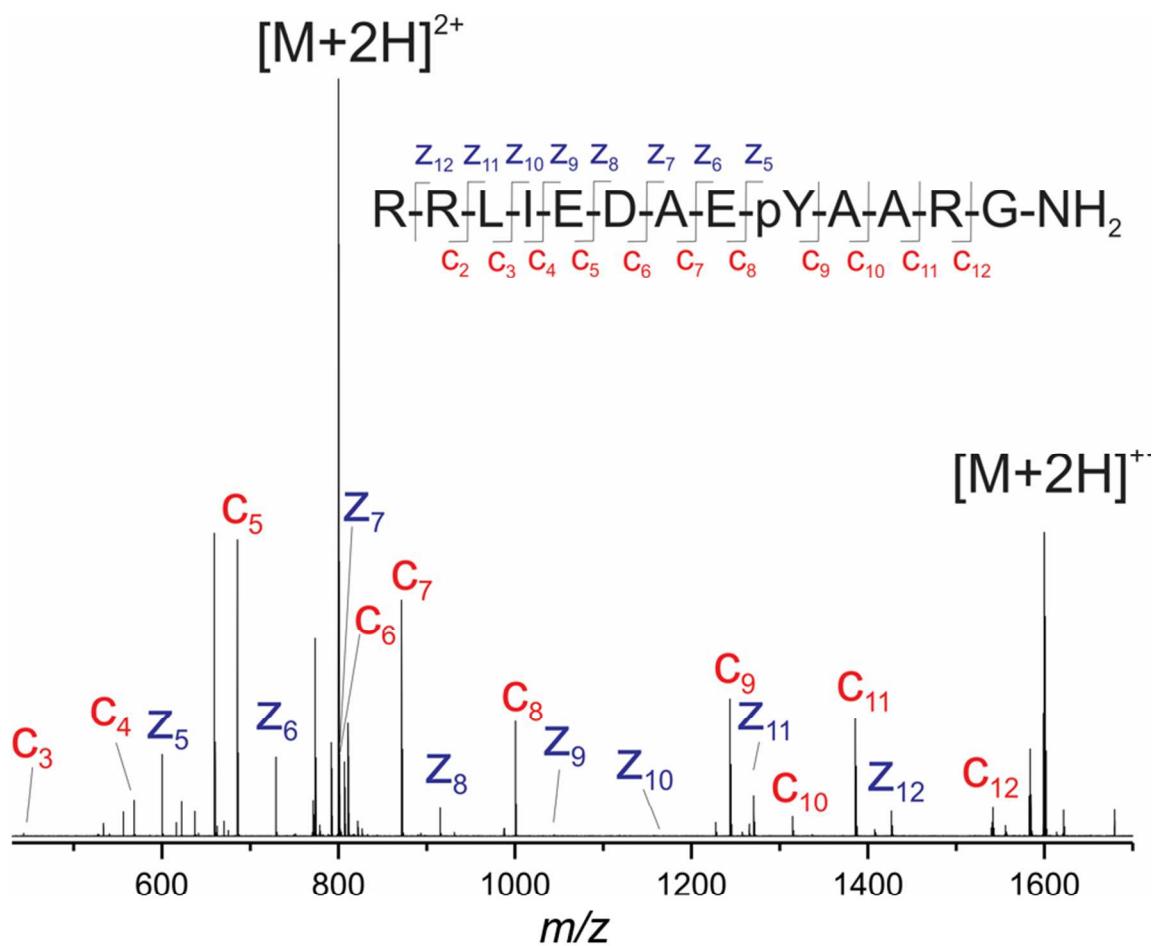
Digestion and LC-MS/MS workflow of HSA: To address the specificity of ECD towards disulfide bond dissociation, a non-reduced tryptic digest of human serum albumin (HSA) (Sigma-Aldrich, Zwijndrecht, Netherlands) was prepared to generate disulfide-linked peptide species. The digest was performed by addition of Lys-C and Trypsin in 1:25 and 1:50 enzyme to protein ratios, respectively, to HSA in NaH₂PO₄/Na₂HPO₄ buffer (pH 7.5) with a final HSA concentration of 0.25 mg/mL. The reaction was incubated overnight and quenched by addition of formic acid to a final concentration of 1% formic acid. 150ng protein digest material was injected and separated on an ultra-HPLC Proxeon EASY-nLC 1000 coupled to the Q Exactive Mass Spectrometer. Reversed-phase separation was performed using a 100 μm inner diameter × 2 cm trap column (packed in-house with ReproSil-Pur C18-AQ, 3 μm) (Dr. Maisch GmbH) coupled to a 50μm I.D. × 50 cm analytical column (packed in-house with Poroshell 120 EC-C18, 2.7 μm) (Agilent Technologies). The mobile phase buffers A and B were water and ACN, respectively, both containing 0.1% FA. A flow rate of 250 nL/min was used with a 40 min gradient (10-30% solvent B within 24 min, 30-100% solvent B within 3 min, 100% solvent B for 2 min, 100-7% solvent B within 1 min and 7% solvent B for 10 min). The 5 most abundant precursors were selected for ECD data dependent fragmentation. For full MS scans, the scan range was 300-2000 m/z at a resolution of 70,000 and the AGC target was set to 3e6. For MS/MS scans, the precursor isolation window was 2.2 m/z and the AGC target was set to 3e6 with maximum injection time of 120ms. MS/MS data were collected in the scan range of 200-2000 m/z at a resolution of 17,500 with 5 uscans, and with a 15s dynamic exclusion filter applied.

Phosphopeptide methods: An Exactive Orbitrap mass spectrometer was modified with an electromagnetostatic ECD cell and was used for recording ECD fragmentation spectra of the phosphopeptides. Like the modification of the Q Exactive mass spectrometers, the ECD cell was mounted in the space between the HCD-cell and the C-trap. For all phosphopeptides, except serine-10-phosphorylated/lysine-14 acetylated Biotin labeled peptide, ECD only fragmentation was

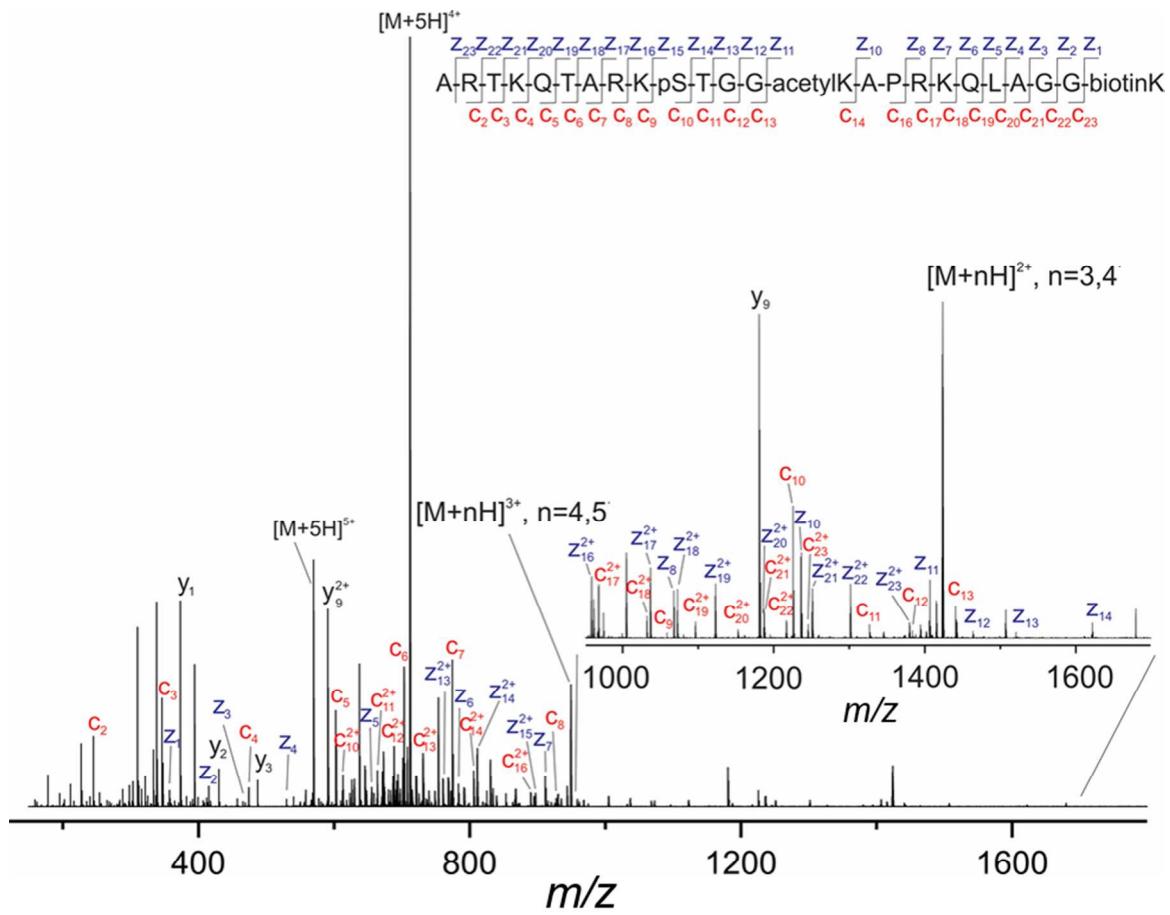
performed and the ions were not transported to the HCD-cell. EChcD was performed for 10-phosphorylated/lysine-14 acetylated Biotin labeled peptide with 10 eV injection energy into the HCD-cell. Tyrosine kinase peptide (RRLIEDAEpYAARG-NH₂) was purchased from American Peptide Co. (Sunnyvale, CA, USA). Other phosphorylated peptides were purchased from Anaspec Inc. (Fremont, Ca, USA). All of the peptides were used without further purification. The samples were prepared using 1:1 water/methanol with 0.1% formic acid to a final concentration of 20-50 µg/mL.



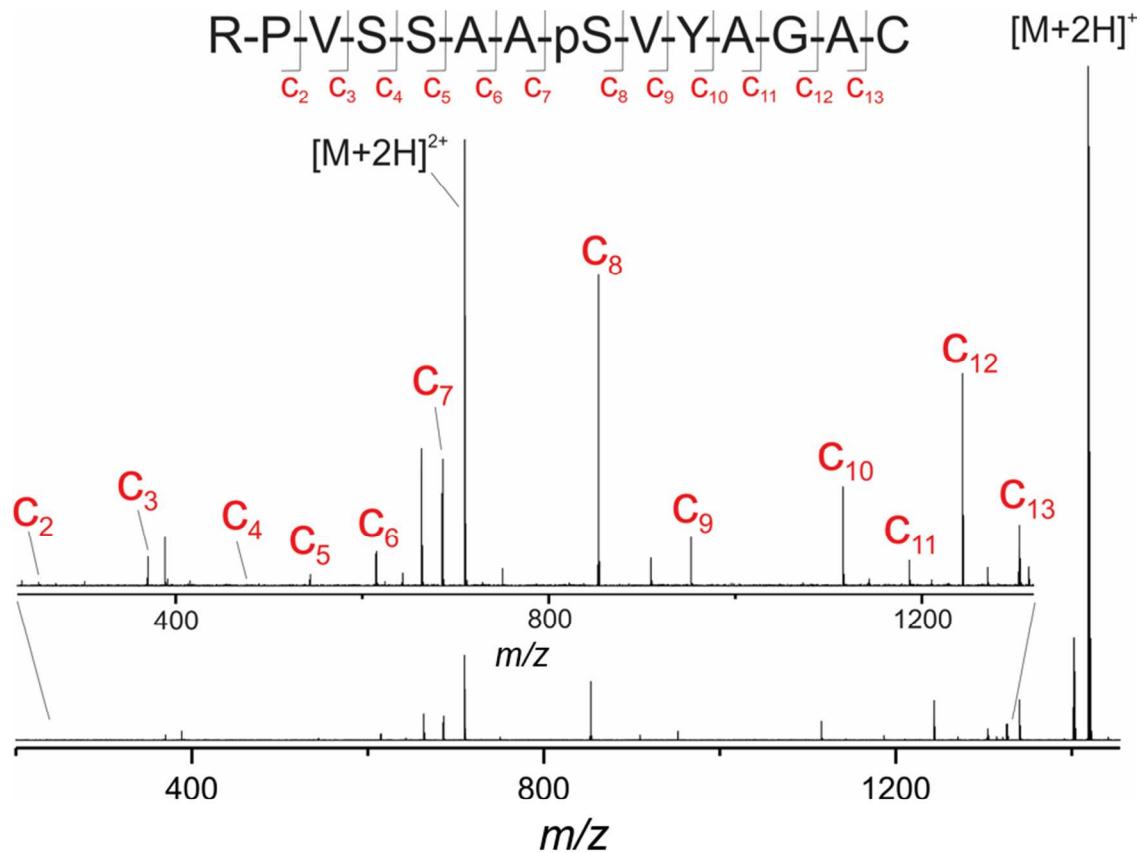
Supplemental Figure 1: The magnetic vector potentials of the device and electron trajectories are shown in a cross-section view of the ECD-cell. The magnetic field guides the electrons stripped from the heated filament to the radial center of the device. Through modifying the electrostatic potentials of E1 through E7, a negative potential well confines the electrons in the axial dimension. Simulated localization of the confined electrons is shown in yellow. The fields were calculated with LORENTZ-EM (Integrated Engineering Software, Winnipeg, Manitoba, Canada).



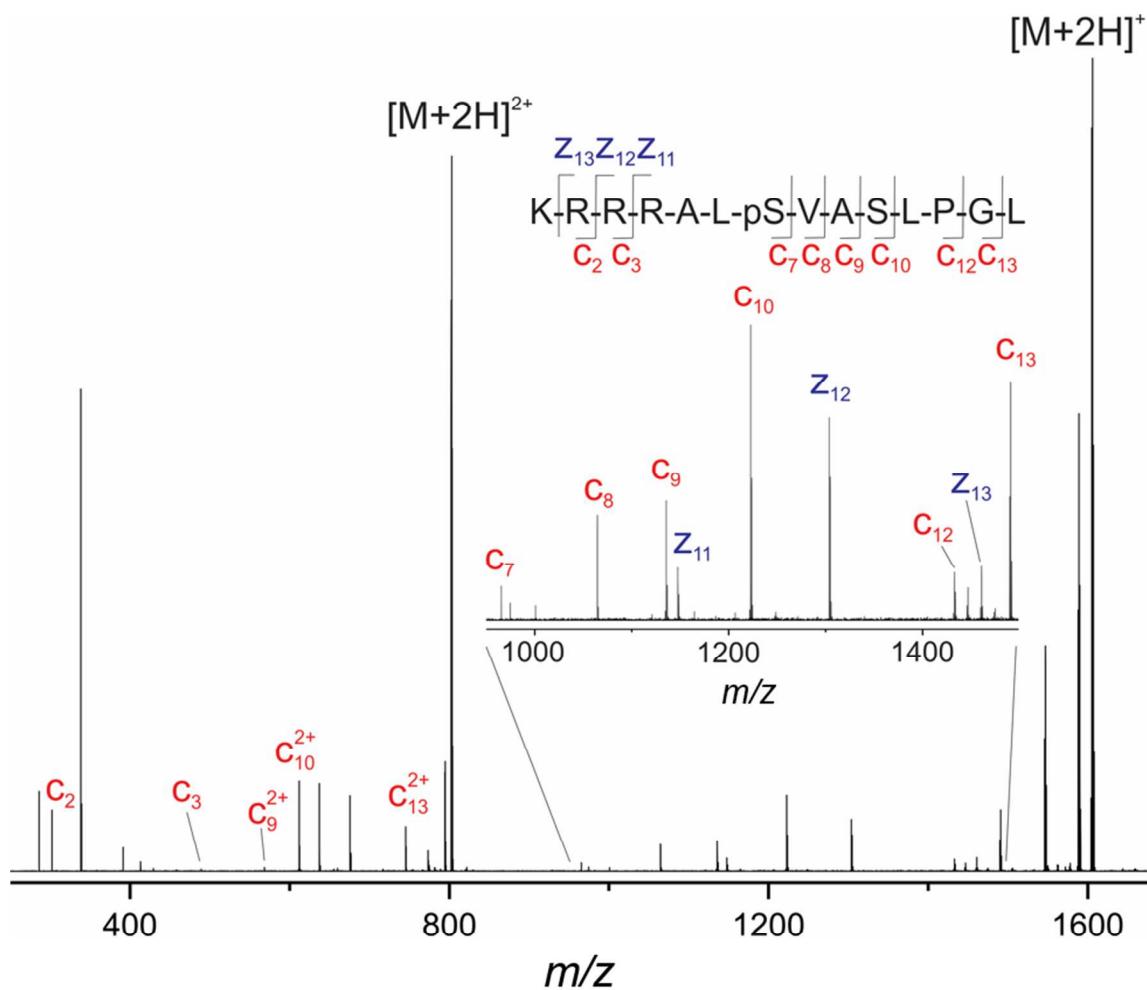
Supplemental Figure 2: The ECD spectrum of a commercially available phosphotyrosine peptide (the sequence is shown in the insert). A range of c and z ion types are visible and provide 100% sequence coverage across the backbone of the peptide. Site localization of the modification site is possible with the z_5 and c_9 ion.



Supplemental Figure 3: The EChcD spectrum of a peptide containing both phosphorylation on serine and acetylation on lysine (the sequence is shown in the insert). Here the spectrum shows a range of different ion types owing to the EChcD fragmentation. Primarily there are *c* and *z* ions that originate from the ECD process, while the *y* ions correspond to HCD fragmentation. The modification is retained for several *c* and *z* ions indicating that EChcD is capable of maintaining labile modifications. Site localization, of both modifications, is possible with the generated *c* and *z* ions.



Supplemental Figure 4: The ECD spectrum of a phosphoserine human keratin peptide with an additional C-terminal cysteine (the sequence is shown in the insert). A range of c ion types are visible and provide high sequence coverage across the backbone of the peptide. Site localization of the modification site is possible with the c_7 and c_8 ion.



Supplemental Figure 5: The ECD spectrum of a serine phosphorylated CK1 peptide substrate [pS7] (the sequence is shown in the insert). A range of c and z ion types are visible across the backbone of the peptide. Site localization of the modification site is possible with the c_7 ion

Electrode Number	Width, mm
E1	1
E2	2
E3	8
E4	4
E5	8
E6	2
E7	1

Supplemental Table 1: The widths of each electrode in the ECD cell modification

Ion Identity	Expected Mass	Measured Mass	ppm Error
b2	254.161	254.159	10.6
c2	271.188	271.184	14.7
c4	496.335	496.329	13.7
b10 ²⁺	600.338	600.336	2.5
c5	624.394	624.388	10.2
c6	752.453	752.445	10.8
c7	899.521	899.513	8.9
c8	1046.589	1046.583	6.4
z9	1078.563	1078.537	23.7
y9	1094.582	1094.568	12.4
c9	1103.611	1103.605	5.8
c10	1216.695	1216.677	15.0

Supplemental Table 2: Matched fragments for $[M+2H]^{2+}$ substance P precursor ion with ECD only activation.

Ion Identity	Expected Mass	Measured Mass	ppm Error
b2	254.161	254.158	11.0
c2	271.188	271.185	11.1
b6 ²⁺	368.217	368.213	10.0
b3	382.256	382.252	10.5
b7 ²⁺	441.751	441.746	10.9
c4	496.335	496.330	10.5
b8 ²⁺	515.285	515.280	10.1
b10 ²⁺	600.338	600.331	11.0
b5	607.368	607.361	11.5
c5	624.394	624.387	11.1
b6	735.426	735.418	10.5
c6	752.453	752.445	10.8
b7	882.494	882.485	10.8
c7	899.521	899.512	10.2
y8	966.487	966.475	12.3
b8	1029.563	1029.552	11.0
c8	1046.589	1046.578	11.0
b9	1086.584	1086.572	11.0
y9	1094.582	1094.570	10.7
c9	1103.611	1103.599	10.9
b10	1191.634	1191.619	12.7
y10	1199.668	1199.655	11.6
c10	1216.695	1216.681	11.3

Supplemental Table 3: Matched fragments for $[M+2H]^{2+}$ Substance P precursor ion with EChcD activation

Ion Identity	Expected Mass	Calculated Monoisotopic <i>m/z</i>	ppm Error
b4	520.259	520.261	3.3
c4	537.285	537.287	3.1
z10 ²⁺	559.358	559.358	0.6
b5	619.327	619.329	3.1
c11 ²⁺	632.876	632.877	1.2
c5	636.354	636.355	1.4
z12 ²⁺	653.397	653.400	3.7
c13 ²⁺	739.940	739.943	3.4
c6	764.449	764.450	0.7
z14 ²⁺	781.966	781.967	0.9
y14 ²⁺	789.976	789.976	1.1
c14 ²⁺	790.466	790.467	1.3
z22 ³⁺	833.126	833.128	2.4
z15 ²⁺	845.996	845.997	2.2
c7	865.496	865.497	1.4
z8	867.565	867.565	0.6
c31 ⁴⁺	869.238	869.239	1.6
y8	883.584	883.584	0.5
z16 ²⁺	902.538	902.539	1.6
y16 ²⁺	910.547	910.548	1.5
c16 ²⁺	911.529	911.531	2.6
z25 ³⁺	942.510	942.514	4.6
y25 ³⁺	947.849	947.853	4.3
z17 ²⁺	959.559	959.561	1.9
c17 ²⁺	961.063	961.064	1.1
y17 ²⁺	967.568	967.570	1.5
c8	978.581	978.581	0.3
z44 ⁵⁺	995.543	995.545	2.7
z35 ⁴⁺	996.053	996.057	4.4
z9	1004.620	1004.628	8.5
z45 ⁵⁺	1018.548	1018.550	2.2
z36 ⁴⁺	1028.068	1028.069	1.3
c28 ³⁺	1035.569	1035.571	2.0
z18 ²⁺	1041.091	1041.092	1.7
z46 ⁵⁺	1044.160	1044.161	1.5
y18 ²⁺	1049.100	1049.102	2.2
c38 ⁴⁺	1053.332	1053.335	3.5
z37 ⁴⁺	1060.082	1060.085	2.0

c75 8+	1063.709	1063.706	2.9
z28 3+	1065.905	1065.907	2.2
z47 5+	1066.776	1066.778	1.8
c19 2+	1074.111	1074.112	1.2
c48 5+	1074.194	1074.200	5.5
c9	1079.630	1079.629	0.6
z38 4+	1088.839	1088.841	1.6
z48 5+	1092.395	1092.397	1.5
y38 4+	1092.844	1092.847	3.0
y48 5+	1095.599	1095.601	1.2
z49 5+	1106.603	1106.604	1.2
y19 2+	1106.614	1106.615	1.2
z59 6+	1107.759	1107.761	1.7
y49 5+	1109.807	1109.808	1.6
c30 3+	1115.962	1115.964	2.5
c20 2+	1117.627	1117.629	1.8
z10	1117.710	1117.709	1.2
c50 5+	1122.422	1122.424	1.1
z60 6+	1124.271	1124.272	0.8
z30 3+	1127.610	1127.611	1.0
z50 5+	1132.222	1132.224	2.1
c10	1136.650	1136.651	0.7
z20 2+	1142.120	1142.123	2.9
z61 6+	1145.778	1145.783	4.4
c51 5+	1148.231	1148.233	1.9
z31 3+	1151.289	1151.292	2.4
c31 3+	1158.648	1158.650	1.5
z62 6+	1164.625	1164.627	1.5
z41 4+	1165.637	1165.639	1.9
y65 6+	1167.295	1167.297	1.7
c52 5+	1171.236	1171.238	1.8
z52 5+	1174.844	1174.846	1.7
c21 2+	1175.140	1175.144	3.3
z42 4+	1179.892	1179.894	2.0
c53 5+	1182.641	1182.643	1.9
y42 4+	1183.897	1183.898	1.3
c42 4+	1185.143	1185.147	3.6
c63 6+	1188.808	1188.810	1.8
c32 3+	1196.992	1196.992	0.0
z21 2+	1198.662	1198.665	2.2

z75 7+	1202.801	1202.803	1.7
c64 6+	1210.315	1210.317	2.1
z43 4+	1212.153	1212.155	2.2
b75 7+	1213.091	1213.090	1.0
c54 5+	1213.861	1213.863	1.5
c75 7+	1215.523	1215.520	2.9
c65 6+	1224.820	1224.824	3.2
c55 5+	1234.070	1234.072	1.5
z66 6+	1238.504	1238.507	2.0
c33 3+	1239.688	1239.691	2.1
c44 4+	1241.685	1241.687	1.5
z55 5+	1243.479	1243.481	1.6
z44 4+	1244.176	1244.180	2.7
y55 5+	1246.683	1246.685	2.2
z22 2+	1249.186	1249.189	2.4
y22 2+	1257.195	1257.197	1.4
c67 6+	1260.509	1260.511	1.9
c11	1264.740	1264.746	4.8
z56 5+	1266.484	1266.493	7.2
z45 4+	1272.933	1272.936	2.2
c57 5+	1274.093	1274.093	0.1
z34 3+	1275.701	1275.705	2.9
c45 4+	1278.452	1278.454	1.8
c68 6+	1283.352	1283.354	1.9
c58 5+	1297.099	1297.101	1.5
z70 6+	1300.538	1300.541	2.6
c35 3+	1301.710	1301.710	0.4
y70 6+	1303.208	1303.210	1.5
z46 4+	1304.948	1304.950	1.5
z12	1305.790	1305.792	1.8
c47 4+	1310.467	1310.470	2.5
c70 6+	1318.711	1318.713	2.1
z71 6+	1321.887	1321.890	2.6
y71 6+	1324.557	1324.561	3.4
z23 2+	1327.237	1327.240	2.5
c59 5+	1329.712	1329.715	2.3
c24 2+	1346.728	1346.730	2.0
z24 2+	1355.747	1355.751	3.0
z48 4+	1365.242	1365.244	1.5

c12	1365.790	1365.794	2.9
y48 4+	1369.247	1369.249	1.3
c61 5+	1375.137	1375.139	1.3
z49 4+	1383.002	1383.003	1.1
z62 5+	1397.349	1397.351	1.4
y62 5+	1400.553	1400.555	1.6
c38 3+	1404.106	1404.111	3.5
z25 2+	1413.261	1413.267	4.6
c75 6+	1417.943	1417.938	2.8
y25 2+	1421.270	1421.276	4.3
z13	1434.830	1434.828	1.4
c51 4+	1435.037	1435.039	1.9
c39 3+	1442.449	1442.451	1.5
z38 3+	1451.450	1451.452	1.6
c64 5+	1452.176	1452.179	2.2
c26 2+	1453.283	1453.287	2.6
z52 4+	1468.303	1468.306	1.7
z26 2+	1477.782	1477.790	5.0
c13	1478.880	1478.878	1.5
c40 3+	1485.135	1485.137	1.6
z66 5+	1486.004	1486.007	2.0
c27 2+	1517.331	1517.333	1.3
c41 3+	1527.821	1527.824	1.7
c68 5+	1539.821	1539.824	1.9
c28 2+	1552.849	1552.852	2.0
c42 3+	1579.855	1579.860	3.6
c14	1579.920	1579.926	3.8
c70 5+	1582.251	1582.255	2.1
z28 2+	1598.353	1598.357	2.2
y28 2+	1606.363	1606.366	2.1
c59 4+	1661.888	1661.891	2.3
c30 2+	1673.439	1673.443	2.5
c15	1693.010	1693.011	0.5
c75 5+	1701.330	1701.325	2.8
c31 2+	1737.468	1737.471	1.5
c32 2+	1794.982	1794.985	1.9
c16	1822.050	1822.056	3.0
c33 2+	1859.029	1859.033	2.1
c17	1921.120	1921.121	0.6
c20	2234.250	2234.251	0.2

Supplemental Table 4: Matched Fragments for ECD of $[M+8H]^{8+}$ Ubiquitin

Ion Identity	Expected Mass	Calculated Monoisotopic <i>m/z</i>	ppm Error
y5	521.272	521.273	2.5
z9 2+	527.278	527.280	3.0
y9 2+	535.288	535.289	2.6
z10 2+	562.797	562.798	2.5
c6	576.262	576.264	2.8
z11 2+	598.315	598.317	2.8
c11 2+	615.809	615.811	3.6
z12 2+	654.857	654.859	2.0
z18 3+	695.029	695.031	1.9
z13 2+	712.371	712.372	2.0
z7	762.391	762.392	1.0
z14 2+	769.392	769.394	1.8
z21 3+	799.101	799.103	1.9
z30 4+	800.160	800.161	1.9
c43 6+	801.251	801.252	1.5
y21 3+	804.441	804.442	1.9
c37 5+	811.824	811.825	1.5
c14 2+	815.404	815.406	3.0
c58 8+	819.177	819.178	0.8
c44 6+	820.422	820.423	0.9
z38 5+	821.412	821.414	2.3
z67 9+	822.553	822.555	2.6
c51 7+	829.441	829.442	1.7
z60 8+	830.069	830.072	3.3
z22 3+	832.784	832.787	3.2
z68 9+	835.118	835.118	0.2
c59 8+	835.308	835.309	1.8
z31 4+	836.927	836.929	2.2
c38 5+	837.632	837.634	1.7
z107 14+	840.316	840.318	2.3
c45 6+	841.771	841.773	2.1
z76 10+	843.352	843.353	1.6
z39 5+	844.029	844.031	2.3

c60 8+	849.686	849.687	1.3
c31 4+	851.444	851.446	2.3
c77 10+	852.860	852.860	0.4
z94 12+	856.638	856.639	1.3
z70 9+	857.349	857.350	1.4
c53 7+	858.023	858.025	1.7
z62 8+	858.080	858.082	2.3
c101 13+	862.395	862.392	3.1
c78 10+	865.670	865.668	1.3
z32 4+	865.684	865.686	2.5
c46 6+	866.283	866.284	2.0
z48 6+	869.449	869.452	2.5
c24 3+	869.770	869.769	0.5
z88 11+	872.752	872.753	1.2
y71 9+	873.467	873.469	2.7
c95 12+	874.471	874.475	4.1
c54 7+	876.458	876.459	1.4
c62 8+	879.833	879.837	4.3
c40 5+	880.458	880.460	1.6
y97 12+	881.899	881.900	1.6
c96 12+	885.146	885.147	1.2
z56 7+	886.044	886.044	1.0
c47 6+	887.632	887.633	1.5
y72 9+	888.696	888.696	0.5
c152 19+	889.584	889.585	1.4
z49 6+	890.957	890.957	0.2
c55 7+	895.178	895.181	2.8
c63 8+	895.845	895.846	1.1
z41 5+	891.255	891.256	1.6
z24 3+	900.143	900.144	0.8
z107 13+	904.878	904.880	2.3
y24 3+	905.483	905.486	3.3
z57 7+	905.623	905.625	1.8
c41 5+	906.267	906.269	2.0
c16 2+	907.963	907.964	1.4
z92 11+	913.685	913.687	1.5

c33 4+	916.482	916.483	1.0
c91 11+	917.861	917.865	4.1
z25 3+	919.150	919.151	0.4
c65 8+	920.105	920.107	1.7
z75 9+	922.713	922.712	0.8
z58 7+	923.923	923.925	2.1
c83 10+	924.497	924.499	2.3
z67 8+	925.246	925.248	2.6
z8	925.454	925.456	1.8
z102 12+	929.586	929.590	4.3
c42 5+	931.886	931.886	0.5
z94 11+	934.423	934.424	1.3
c58 7+	936.059	936.059	0.8
z76 9+	936.946	936.947	1.6
z51 6+	936.981	936.982	1.1
z35 4+	938.467	938.471	3.8
y51 6+	939.651	939.653	2.3
c85 10+	944.505	944.507	1.9
c77 9+	947.510	947.511	0.4
z60 7+	948.506	948.509	3.4
z44 5+	950.696	950.699	3.4
c59 7+	954.493	954.495	1.8
c17 2+	957.497	957.497	0.3
z52 6+	958.330	958.332	1.7
z88 10+	959.926	959.927	1.3
c43 5+	961.300	961.301	1.5
c78 9+	961.743	961.742	1.2
z70 8+	964.392	964.393	1.4
c51 6+	967.513	967.514	1.7
z61 7+	968.086	968.087	1.4
z36 4+	970.491	970.493	2.0
c60 7+	970.926	970.927	1.3
z45 5+	973.701	973.703	2.5
c27 3+	974.477	974.479	1.9
c79 9+	975.976	975.977	1.0
z17 2+	977.519	977.520	1.1

z62 7+	980.519	980.521	2.3
y71 8+	982.524	982.527	2.7
c44 5+	984.305	984.306	0.8
z27 3+	985.516	985.517	1.7
c61 7+	987.081	987.083	2.0
c52 6+	989.020	989.020	0.1
z46 5+	991.107	991.110	2.8
z37 4+	992.249	992.250	1.3
c53 6+	1000.859	1000.861	1.7
c62 7+	1005.380	1005.384	4.3
c45 5+	1009.924	1009.926	2.1
z55 6+	1012.200	1012.201	0.8
c37 4+	1014.528	1014.529	1.4
c9	1018.460	1018.462	2.0
c54 6+	1022.366	1022.368	1.5
z28 3+	1023.858	1023.860	2.0
z38 4+	1026.513	1026.516	2.3
c83 9+	1027.107	1027.109	2.3
z94 10+	1027.764	1027.766	1.3
z56 6+	1033.550	1033.551	1.0
c46 5+	1039.338	1039.340	1.9
z18 2+	1042.040	1042.042	1.8
z48 5+	1043.138	1043.140	2.5
c38 4+	1046.788	1046.790	1.7
c65 7+	1051.405	1051.407	1.7
z9	1053.550	1053.550	0.0
z57 6+	1056.393	1056.395	1.7
c47 5+	1064.957	1064.958	1.5
z49 5+	1068.946	1068.947	0.3
c39 4+	1072.050	1072.052	1.4
c30 3+	1082.890	1082.892	2.3
c40 4+	1100.321	1100.323	1.5
z60 6+	1106.423	1106.426	3.4
c59 6+	1113.408	1113.410	1.8
z10	1124.590	1124.586	3.8
y51 5+	1127.380	1127.382	2.3

c41 4+	1132.582	1132.584	2.0
z20 2+	1134.101	1134.104	2.9
c31 3+	1134.923	1134.926	2.4
z32 3+	1153.909	1153.912	2.4
c51 5+	1160.814	1160.816	1.7
c21 2+	1171.592	1171.595	2.4
c43 4+	1201.373	1201.375	1.5
y21 2+	1206.158	1206.160	1.8
c22 2+	1207.111	1207.112	1.2
z55 5+	1214.439	1214.440	0.7
c11	1230.610	1230.613	2.4
c23 2+	1235.622	1235.624	2.0
z12	1308.710	1308.712	1.4
c12	1344.650	1344.658	5.7
z24 2+	1349.711	1349.712	0.7
c13	1443.720	1443.724	2.9
c27 2+	1461.213	1461.215	1.9
c14	1629.800	1629.806	3.5
c15	1686.820	1686.829	5.1

Supplemental Table 5: Matched Fragments for ECD of $[M+20H]^{20+}$ myoglobin